ANTIBODIES TO LA CROSSE VIRUS IN EASTERN CHIPMUNKS IN INDIANA NEAR AN *AEDES ALBOPICTUS* POPULATION

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ABSTRACT. Blood samples of 34 Eastern chipmunks trapped in the vicinity of an *Aedes albopictus* population near New Alsace, IN, were tested for neutralizing antibodies to La Crosse (LAC) virus and other California group viruses. Two samples were positive for LAC antibodies. Analysis of mosquito bloodmeals demonstrated that both *Ae. albopictus* and *Ae. triseriatus* from the site fed on chipmunks. This note documents the first record of LAC antibodies in sylvan rodents from Indiana, the presence of LAC virus in the vicinity of *Ae. albopictus* and that wild *Ae. albopictus* feed on Eastern chipmunks.

The Asian tiger mosquito, *Aedes albopictus* (Skuse), was first identified in the United States near Houston, TX, in 1985 (Sprenger and Wuithiranyagool 1986). Since then the species has spread throughout most of the southern and eastern USA (Nawrocki and Hawley 1987). Grimstad et al. (1989) showed that *Ae. albopictus* can transmit La Crosse (LAC) virus orally, and Tesh (1980) has demonstrated transovarial transmission, thus making the species a potential vector for LAC virus if it feeds on viremic vertebrate hosts in the wild. *Aedes albopictus* is diurnal, and it feeds on a wide variety of mammal species (Hawley 1988).

We conducted serological surveys of Eastern chipmunks (*Tamias striatus* Linn.) at 2 sites: one near New Alsace, Dearborn Co., IN, where there is an established population of *Ae. albopictus* at a used tire pile, and a second at Festge Park, near Madison, Madison Co., WI, to determine the prevalence of LAC antibodies in chipmunks at a known focus. At the New Alsace site we also made mosquito collections to verify the presence and relative abundance of *Ae. albopictus* using hand-held vacuum aspirators for landing-biting counts. Mosquito collections were made at the periphery of the tire pile and in the woods 100–500 m to the east. Unfortunately, permission was not granted to make collections within the tire pile. The results of the mosquito collections are listed in Table 1.

A ground aspirator collection (Nasci 1981) was also made at the south edge of the tire pile on August 10, 1989, and engorged mosquitoes were sent to the Centers for Disease Control (CDC), Fort Collins, CO, where blood was identified by precipitin tests and ELISA. In this collection, six *Ae. albopictus* were engorged and 2 (33%) of these had fed on sciurids. Sixty-six of the *Ae. triseriatus* (Say) were engorged, and 37 (56%) of the blood-meals were identified as sciurid (Harold Savage and Mark Niebylski, unpublished data). Because Eastern chipmunks were the most abundant sciurid near the tire pile, most of the sciurid blood can be attributed to that species. *Aedes triseriatus* (606 females in 67 pools and 494 males in 21 pools) from that collection were also screened by plaque assay on Vero cells for LAC virus. None were positive for LAC virus (Chester G. Moore, unpublished data).

Eastern chipmunks were trapped at New Alsace during June and August 1989 and July 1990, and at Festge Park in July 1989. Chipmunks were trapped in Sherman live-traps, anesthetized with Metofane® (Pitman-Moore, Inc.), bled by cardiac puncture, marked with an ear tag (National Band and Tag Co.) and released. In the field the blood samples were centrifuged and placed on ice. Long term storage was at −20°C.

Serum dilution neutralization tests (Pantuwatana et al. 1972) were performed with an Indiana isolate of LAC virus (Pinger et al. 1983) and the prototype strains of 2 other California serogroup viruses, Jamestown Canyon (JC) virus and trivittatus (TVT) virus. A serum sample was identified as having a specific antibody type if its highest titer was at least 4 times that of the next highest heterologous titer.

One (7%) of 14 chipmunks, an adult male, collected at New Alsace on June 26, 1989, had LAC specific antibodies at a titer of 1:128. In August 1989, 7 chipmunks were captured at New Alsace, and all were sero-negative. On July 10, 1990, one (8%) of 13 chipmunks was sero-positive. This chipmunk had a nonspecific antibody response with a titer of 1:128 (LAC), 1:64 (JC) and 1:32 (TVT). This was an unmarked adult male with an injured ear where an eartag may have been removed. At the site in Wisconsin, 5 (36%) of 14 chipmunks had California serogroup antibodies with a geometric mean LAC antibody titer of 1:169. Three were typed as LAC, and the

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Table 1. Mosquito collections from New Alsace, IN. Landing-biting collections were made by 1–3 individuals. The duration is the sum of minutes of collection time by all collectors at a site on a given date. Ground aspirator collections were made in undergrowth at the rear of the tire pile. The southeast collection site was approximately 200 m from the tires, and the southwest collection site was approximately 25 m from the tires.

Date	Area and species	Number (♀/♂)	Duration	No. collected/h
Aug. 3, 1989	Southeast (landing-biting)			
	Ae. albopictus	9/5	120	7
	Ae. triseriatus	31/1	120	16
	Southwest (landing-biting)			
	Ae. albopictus	27/8	180	12
	Ae. triseriatus	71/1	180	24
Aug. 10, 1989	Southwest (ground aspiration)			
	Ae. albopictus	7/12	90	13
	Ae. triseriatus	354/442	90	531
July 11–21, 1990	Southeast (landing-biting)			
	Ae. albopictus	0	45	0
	Ae. triseriatus	1/0	45	1
	Southwest (landing-biting)			
	Ae. albopictus	1/0	30	2
	Ae. triseriatus	55/0	30	110
July 31, 1990	On site (landing-biting)[1]			
	Ae. albopictus	0	52	0
	Ae. triseriatus	497/0	52	573
Sept. 13, 1990	On site (landing-biting)[1]			
	Ae. albopictus	13/0	75	10
	Ae. triseriatus	512/0	75	410

[1] These collections were made by Brad Foster of the Indiana Board of Health at the south end of the New Alsace tire pile.

remaining 2 had significant cross-reactions. In previous chipmunk surveys in Wisconsin, the proportion of LAC sero-positive chipmunks varied from 11 to 100% at different sites (Moulton and Thompson 1971, Gauld et al. 1974).

The tire pile at New Alsace, IN, that harbors the *Ae. albopictus* population is less than 600 m from the site of capture of the positive chipmunks. The dominant mosquito species at the tire pile was *Ae. triseriatus*, the native vector of LAC virus in the USA. Consequently, the presence of LAC sero-positive chipmunks does not indicate that *Ae. albopictus* is transmitting LAC virus in the wild, only that it is now found in a local setting where the virus is demonstrated to be present. The LAC antibody positive sera from New Alsace are the first records of LAC virus infection in sylvan rodents from Indiana. Whether the expanding populations of *Ae. albopictus* will affect LAC antibody prevalence and LAC virus transmission to chipmunks and humans remains an important question.

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REFERENCES CITED

Gauld, L. W., R. P. Hanson, W. H. Thompson and S. K. Sinha. 1974. Observations on a natural cycle of La Crosse virus (California Group) in southwestern Wisconsin. Am. J. Trop. Med. Hyg. 23:983–992.

Grimstad, P. R., J. F. Kobayashi, M. Zhang and G. B. Craig, Jr. 1989. Recently introduced *Aedes albopictus* in the United States: potential vector of La Crosse virus (Bunyaviridae: California serogroup). J. Am. Mosq. Control Assoc. 5:422–427.

Hawley, W. A. 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 4(Suppl. 1): 1–39.

Moulton, D. W. and W. H. Thompson. 1971. California group virus infections in small, forest-dwelling mammals of Wisconsin. Am. J. Trop. Med. Hyg. 20:474–482.

Nasci, R. S. 1981. A lightweight battery-powered aspirator for collecting resting mosquitoes in the field. Mosq. News 41:808–811.

Nawrocki, S. J. and W. A. Hawley. 1987. Estimation of the northern limits of distribution of *Aedes albo-*

pictus in North America. J. Am. Mosq. Control Assoc. 3:314–317.

Pantuwatana, S., W. H. Thompson, D. M. Watts and R. P. Hanson. 1972. Experimental infection of chipmunks and squirrels with La Crosse and trivittatus viruses, and biological transmission of La Crosse virus by *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 21:476–481.

Pinger, R. R., P. R. Grimstad, M. J. Sinsko and C. H. Calisher. 1983. Isolation of La Crosse and other arboviruses from Indiana mosquitoes, 1961–1982. Mosq. News 43:459–463.

Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. J. Am. Mosq. Control Assoc. 2:217–219.

Tesh, R. B. 1980. Experimental studies on the transovarial transmission of Kunjin and San Angelo viruses in mosquitoes. Am. J. Trop. Med. Hyg. 29:657–666.